Scientific Reports

https://doi.org/10.1038/s41598-026-47121-5

Article in Press

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Received: 5 February 2026

Accepted: 30 March 2026

Published online: 08 April 2026

Cite this article as: Tiritelli R., Sartore A., Zavatta L. *et al.* Opposite seasonal and spatial dynamics of DWV-A and DWV-B suggest distinct transmission pathways in managed honey bees (*Apis mellifera* L.) colonies. *Sci Rep* (2026). https://doi.org/10.1038/s41598-026-47121-5

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Opposite seasonal and spatial dynamics of DWV-A and DWV-B suggest distinct transmission pathways in managed honey bees (*Apis mellifera* L.) colonies

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Abstract

Deformed wing virus (DWV) is a major viral pathogen of *Apis mellifera*, existing mainly as two genotypes, DWV-A and DWV-B, which differ in transmission dynamics and virulence. This study presents a three-year national molecular surveillance (2021–2023) of Italian honey bee colonies to investigate the spatio-temporal distribution of both variants in relation to apiary density, geographical gradients, and land-use patterns. Quantitative PCR (qPCR) and Bayesian spatio-temporal models were applied to assess viral prevalence and environmental predictors. DWV-B was the dominant variant (overall 73.7%) and displayed a marked autumnal peak in November, followed by a winter decline. In contrast, DWV-A showed a complementary trend, peaking in summer and decreasing with apiary density, suggesting an environmentally mediated transmission pathway. Spatial analysis revealed higher DWV-B prevalence in southern and insular regions, whereas DWV-A predominated in central and northeastern regions. Land-use effects further indicated that DWV-B is linked to anthropogenic landscapes with intensive beekeeping, while DWV-A is associated with more heterogeneous environments. These findings highlight distinct ecological dependencies between DWV variants: DWV-B is probably more *Varroa*-associated, colony-driven virus favoured by warm, stable climates, while DWV-A reflects diffuse environmental persistence. Integrating climatic and management factors is essential to predict DWV epidemiological shifts under global change.

Introduction

The deformed wing virus (DWV) is one of the most frequently detected and extensively studied viruses infecting the western honey bee (*Apis mellifera*) [1,2]. This virus is a positive single-stranded RNA (+ssRNA) virus, belonging to the order Picornavirales, family Iflaviridae, genus *Iflavirus* [3].

DWV has been closely linked to colony declines [4–7], particularly when high viral loads lead to clear symptomatic infections. In individual bees, DWV causes wing deformities, reduced lifespan, impaired foraging behaviour, and weakened immunity [3,4,8]. At the colony level, these effects can culminate in population collapse, especially when compounded by high *Varroa destructor* infestation levels [9,10]. The spread of *Varroa* has been strongly linked to an increased prevalence and load of DWV in the colony members [2,11]. This is primarily due to the mite's role as an exceptionally efficient biological vector, injecting viral particles directly into developing pupae and adults while feeding [12–14]. Consequently, *Varroa*-mediated transmission is considered the primary horizontal route of DWV infection [14]. However, additional transmission pathways exist, including trophallaxis within the colony, the feeding of virus-contaminated food to larvae, and oral–faecal transmission, including

cannibalism [13–15]. DWV can also be spread through the sharing of infected food such as honey, pollen, and royal jelly [14,16,17], and through foraging flowers that may be visited by other pollinators, creating interspecies transmission opportunities [18–20]. Vertical transmission is also possible, occurring via infected queens laying virus-laden eggs [21], and potentially through sexual transmission during mating flights between infected breeders [22].

The DWV presence has been reported globally and ubiquitously in honey bee colonies across all continents [1,23–25]. Beyond honey bees, DWV has also been detected in a broad range of wild and managed pollinators, as well as in other insect taxa and arthropods [26]. This raises significant concerns about potential viral spillover events, cross-species transmission, and the broader ecological consequences for pollinator communities.

Initially described as a single dominant genotype, DWV is now recognised as a complex of at least three major master variants: DWV-A (the originally characterised strain), DWV-B (historically referred to as VDV-1), and the less frequently reported and highly virulent DWV-C [27–29]. A fourth variant (DWV-D) was identified from *A. mellifera* in Egypt but has not been subsequently detected [30]. Among these, DWV-A remains the most commonly reported globally [25,31], suggesting it has been circulating for a longer time. In contrast, DWV-B was first detected in *V. destructor* in 2004 [32]. The absence of earlier records likely reflects limited surveillance and underestimation rather than true absence. Since its discovery, DWV-B has been reported in an increasing number of countries, and a recent global trend shows its progressive replacement of DWV-A in many regions [2]. DWV-B has been associated with

higher viral loads, increased virulence, and elevated fitness, leading to a shift in viral population structure across Europe and other parts of the world [2,33]. Recent studies have shown that DWV-B, unlike DWV-A, can actively replicate within *V. destructor*, potentially giving it a transmission advantage [34]. Recombinant genomes and evolving host–vector–virus interactions further complicate the DWV landscape, suggesting an ongoing adaptive evolution in response to ecological and anthropogenic pressures [35–37].

Fragmented epidemiological data are available in Italy, and increasing attention has been given to the distribution of DWV in honey bee colonies [38–41], as well as their occurrence in honey bee predators [42,43] and other bee and insect hosts [16,44–46]. Recent reports have highlighted the growing presence of DWV-B in Italian populations of *A. mellifera* [47].

The aim of this study was to determine the prevalence and abundance of DWV-A and DWV-B variants within a nationwide three-year monitoring program across Italian honey bee colonies. The spatial and spatio-temporal distributions of prevalence for each variant were analysed separately, assessing the impact of land-use covariates through geostatistical models. These data aim to provide an updated overview of the current epidemiological status of DWV variants in Italian apiary into the agricultural ecosystem and to improve the understanding of the transmission dynamics and spread of DWV variants in managed bee populations.

Results

Descriptive Analyses

A total of 12028 samples were collected and analysed across all Italian regions from 380 apiaries monitored over the three-year national surveillance program. The total number of samples received and analysed (n = 12028) was slightly lower than the number expected based on the colonies monitored (n = 13256) (Table S1). This discrepancy was due to several factors: i) concerns about hive disturbance in regions with the coldest climates during November; ii) missing samples from technicians or beekeepers due to issues unrelated to the BeeNet project; and iii) colony losses caused by factors other than pathogens (including DWV) or *Varroa* pressure. The specific number of samples collected each year, as well as their regional and provincial distribution, is detailed in Table S2.

Overall, 88.66% of the samples tested positive for DWV, as shown in Table 1, with a lower prevalence of the DWV-A variant (15.02%) and a higher prevalence of the DWV-B variant (74.27%). Throughout the three-year monitoring period, the prevalence of both DWV variants remained relatively stable, except in the second year, when DWV-B prevalence declined to 65.41% while DWV-A increased to 19.17%. The viral load also differed between variants, with a mean abundance of $3.37\times 10^{11} \pm 3.68 \times 10^{13}$ for DWV-A and $2.59 \times 10^{8} \pm 4.86 \times 10^{9}$ for DWV-B. Over the three years, DWV-B abundance remained stable, whereas DWV-A showed a decreasing trend, reaching a mean value of $2.88 \times 10^{8} \pm 6.17 \times 10^{9}$ in the third year.

Table 1. Pathogen prevalence (with 95% confidence intervals) and mean copy number (± standard deviation) for each variant. Italy, 2021-2024.

Pat	Sampling period	N° of analysed	N° of positive	Prev	95% CI	Mean copy number	Standard deviation

		samples	**samples**				
DWV-A	Total (2021-2024)	12028	1807	15.02%	[14.29%, 15.56%]	3.37×10^{11}	3.68×10^{13}
	1st year (2021-2022)	3891	472	12.13%	[11.14%, 13.19%]	1.04×10^{12}	6.46×10^{13}
	2nd year (2022-2023)	4195	804	19.17%	[18%, 20.38%]	2.59×10^{9}	6.82×10^{10}
	3rd year (2023-2024)	3942	531	13.47%	[12.44%, 14.57%]	2.88×10^{8}	6.17×10^{9}
DWV-B	Total (2021-2024)	12028	8933	74.27%	[72.95%, 74.52%]	2.59×10^{8}	4.86×10^{9}
	1st year (2021-2022)	3891	3125	80.31%	[79.03%, 81.53%]	1.08×10^{8}	6.00×10^{9}
	2nd year (2022-2023)	4195	2744	65.41%	[63.96%, 66.84%]	3.84×10^{8}	5.44×10^{9}
	3rd year (2023-2024)	3942	3064	77.73%	[76.4%, 79%]	2.03×10^{8}	2.21×10^{9}

Note: The sampling period is reported according to reference years, as each sampling year begins in the fall of one year and ends in the spring of the following year.

At the regional level, all regions showed an overall DWV prevalence exceeding 75%, although the distributions of the two variants varied geographically, as illustrated in Figure 1. The highest prevalences of DWV-A were observed in Umbria (28.77%), Lazio (24.90%), and Campania (23.28%). In contrast, DWV-B was most prevalent in Calabria (88.32%), Basilicata (85.61%), and Apulia (84.82%). When considering broader geographic areas, the southern regions exhibited the highest DWV-B prevalence (81.24%), followed by the islands (76.42%), the central (72.18%), the northwest (72.30%), and the northeast (69.88%). Central Italy also showed the highest DWV-A prevalence (19.44%), followed by the northeast (14.84%), the south (14.77%), the islands (14.66%), and the northwest (13.09%).

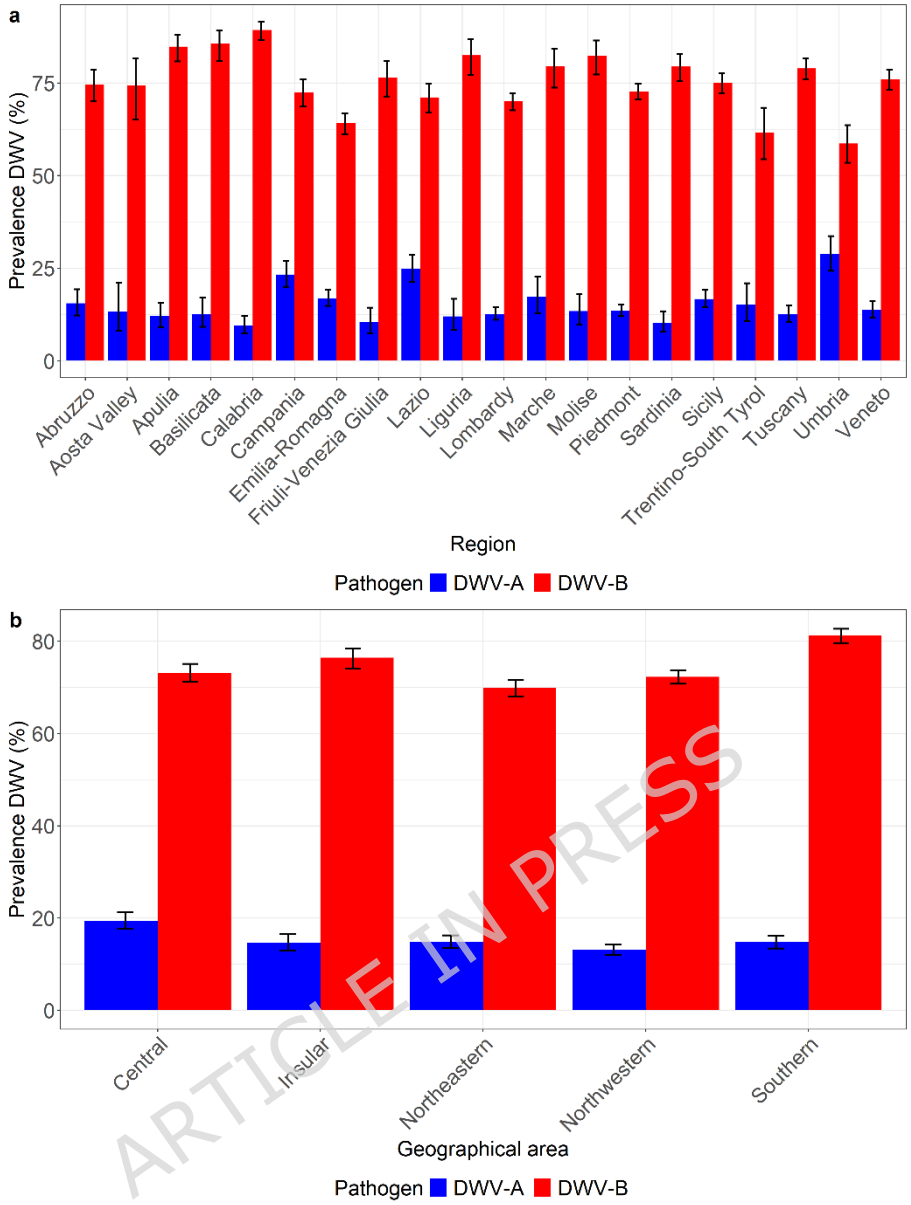

Figure 1. Mean prevalence of DWV-A and DWV-B across Italian regions (a) and different geographical areas (b). Prevalence is indicated as a percentage. Error bars represent the 95% confidence intervals (CI). Italy, 2021-2024.

Seasonal trends in DWV-A and DWV-B showed opposite patterns throughout the sampling months, as depicted in Figure 2. The prevalence and mean abundance of DWV-A increased during the early sampling period, reaching a peak in mid-season before declining towards the end of the year. In contrast, DWV-B displayed an inverse trend, with lower values in the early months and a progressive rise in prevalence and abundance later in the season.

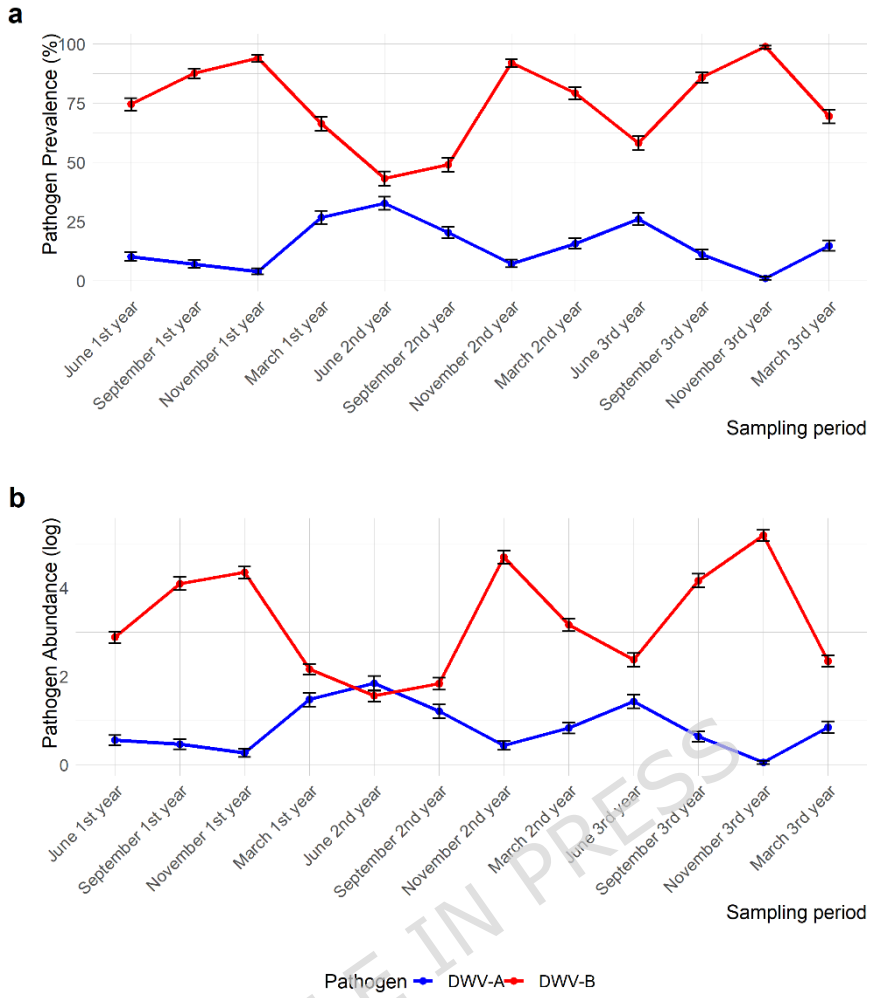

Figure 2. Seasonal trends in the prevalence of DWV-A and DWV-B (a) and in the mean abundance of the two variants (b) throughout the sampling period. Prevalence is expressed as a percentage, while abundance is shown on a logarithmic scale. Error bars represent the 95% confidence intervals (CI) in prevalence and the standard deviation (±SD) in abundance. Italy, 2021-2024.

Co-presence of the two virus variants (DWV-A and DWV-B) was not observed in any single colony. However, both variants were detected within the same apiary, albeit in different colonies, with the following prevalences: 283 out of 1304 apiaries (21.7%, 95% CI [19.5%, 24%]) in the first year; 370 out of 1403 (26.4%, 95% CI [24.1%, 28.7%]) in the second year; and 242 out of 1330 (18.2%, 95% CI [16.2%, 20.4%]) in the third year.

Bayesian Geostatistical and Spatio-Temporal Models

Figure 3 shows the maps of the posterior predicted probabilities of infection from the Bayesian geostatistical spatio-temporal models for DWV-A and DWV-B, respectively. The scale of the legend is the same, allowing them to be compared. Maps of uncertainty, i.e. of the standard deviations, are reported in Figures S1 and S2. A clear opposite trend between the two pathogens is observed, with DWV-A reaching its seasonal peak in September of 1st year and June of 2nd and 3rd year, so between summer and autumn, while DWV-B reaches the highest prevalence always in November. This turnover is also visible at the local level, with areas such as Apulia, that tend to have a higher prevalence of DWV-A compared to DWV-B.

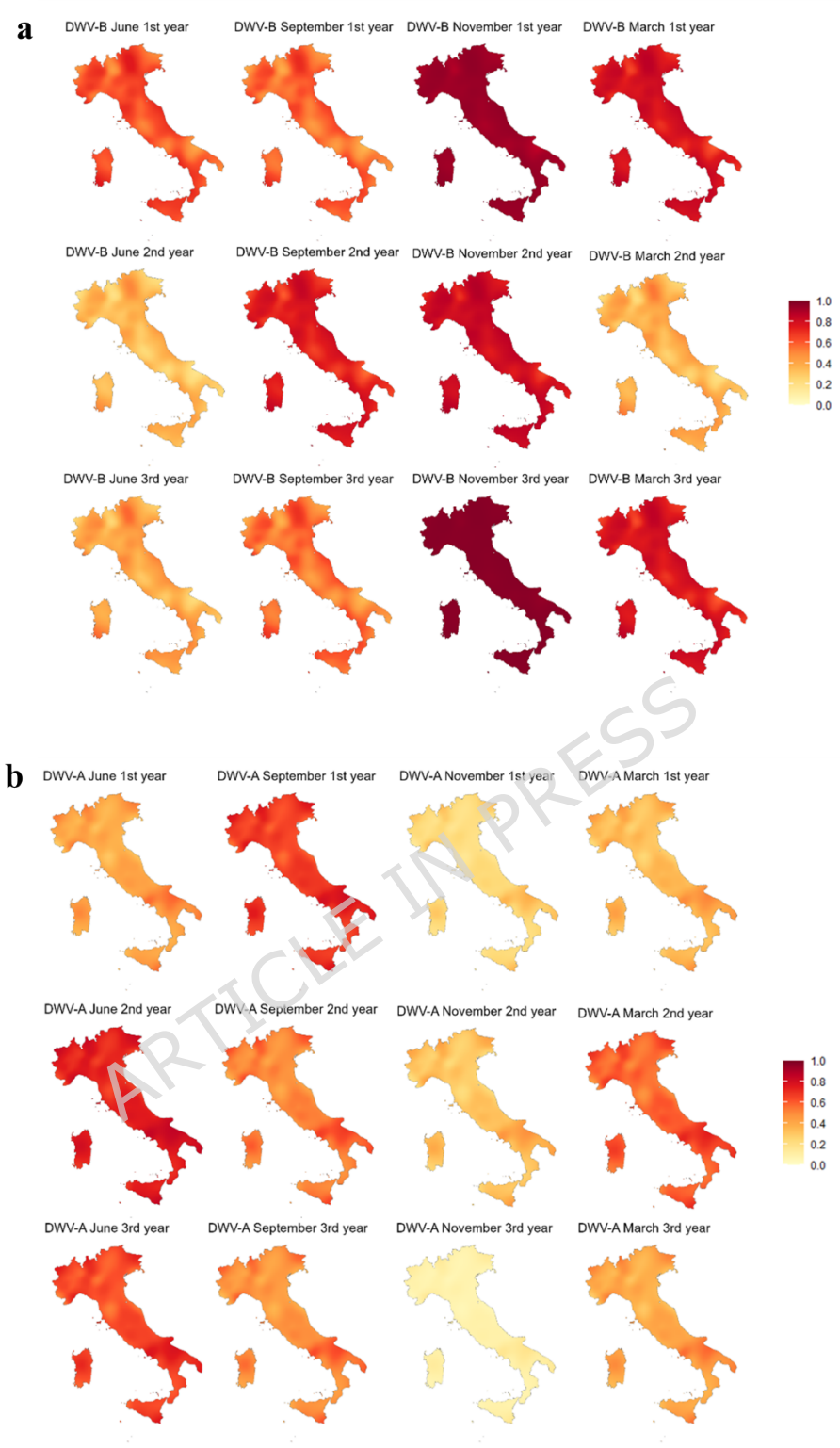

Figure 3. Maps of posterior predicted probability of infection for DWV-B (a) and for DWV-A (b) from the Bayesian spatio-temporal model. Italy, 2021-2024.

The contrasting temporal trends of the two pathogens are even more evident in Figure 4, where the posterior mean of the time random effects of the spatio-temporal models - modelled as a conditional autoregressive process - are plotted.

Maps produced from geostatistical models, i.e. without considering time dimension, are reported, as a sensitivity analysis, in Figures S3 and S4.

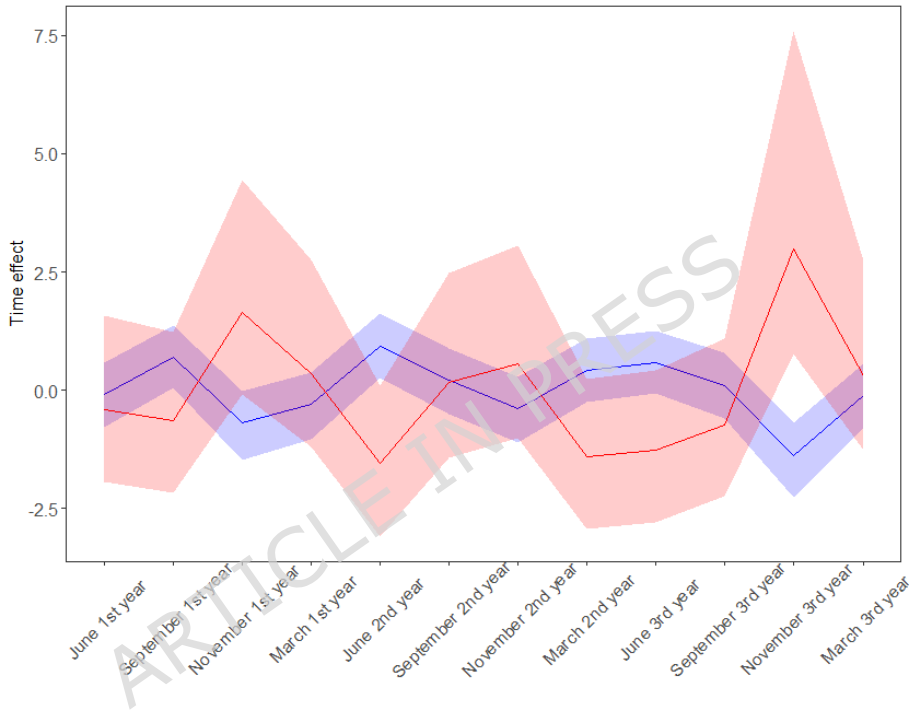

Figure 4. Posterior distribution of the time random effect of the Bayesian spatio-temporal model, for DWV-A (blue) and DWV-B (red) with 95% Credibility Intervals. Italy, 2021-2024.

Bayesian Spatio-Temporal Models with covariates

In Table 2, posterior mean estimate of the regression coefficients of the Bayesian spatio-temporal model with covariates are reported, together with 95% Credibility interval (95% CrI). As mentioned in the methods section, all coefficients measuring the soil composition should be interpreted relative to a change in the corresponding land-cover specific category with respect to the

percentage of heterogeneous agricultural areas. For example, an increase in arable land with respect to heterogeneous agricultural areas is associated with a decrease in the prevalence of DWV-A. The opposite trends between the two variants are again evident, with coefficients having opposite directions for DWV-A and DWV-B. The credibility intervals are generally very narrow, indicating high precision in the regression coefficient estimates, although effect sizes are very close to zero. This does not apply to the covariate density of apiaries, which, on the contrary, shows a clear and net effect, decreasing the prevalence of DWV-A and increasing that of DWV-B.

Table 2. Posterior mean and 95% Credibility intervals of regression coefficient of the Bayesian spatio-temporal models with covariates, for DWV-A and DWV-B.

Spatio-Temporal Model		
Covariate	**Coefficient (95% CrI)**	
	DWV-A	**DWV-B**
Density of apiaries	-0.444 (-0.553, -0.327)	1.393 (1.135, 1.625)
Arable lands*	-0.029 (-0.046, -0.014)	0.079 (0.048, 0.110)
Artificial surfaces*	0.012 (-0.003, 0.026)	-0.033 (-0.061, -0.005)
Permanent crops*	0.018 (0.001, 0.036)	-0.054 (-0.093, -0.009)
Forest, seminatural and wet areas*	0.014 (0.005, 0.022)	-0.037 (-0.054, -0.019)

*Coefficients should be interpreted wrt Heterogeneous agricultural areas.

Discussion

The results of this large-scale national surveillance clearly indicate that DWV-B is the dominant and most abundant variant circulating among Italian honey bee colonies, showing an average prevalence of 73.7%, compared with 14.9% for DWV-A. This pattern reflects a global epidemiological shift already reported in

several European countries and beyond, where DWV-B is progressively replacing DWV-A [2,33,37,48,49]. The dominance of DWV-B can be attributed to its enhanced fitness and replication efficiency, as well as its ability to actively replicate within the *Varroa* mite [34]. This feature grants DWV-B a substantial ecological advantage over DWV-A, allowing more efficient transmission within and between colonies. Besides, the consistent association between DWV-B prevalence and high *Varroa* infestation levels reinforces the view that this genotype has become tightly coupled with *Varroa*-mediated transmission routes, which are now considered the main drivers of DWV epidemiology in managed honey bee populations [5,6,25].

Interestingly, none of the samples analysed in this study tested positive for both DWV variants, suggesting a lack of clear evidence for recombinant forms between DWV-A and DWV-B. Nevertheless, an increasing number of recent studies have reported the occurrence of recombinant viruses between these two DWV variants in natural populations [7,50–54]. It has also been proposed that such recombinants may exhibit higher virulence than either DWV-A or DWV-B alone, particularly during the pupal stage of honeybees [55,56]. The absence of mixed infections in the analysed samples is consistent with previous findings [7], and supports the reliability of the methodology used in this study. In fact, although no viral sequencing was performed in this investigation, the applied approach is capable of detecting the DWV-A/B recombinants described so far in the literature, since it amplifies the 3′ genomic region derived from DWV-A, which includes the *RNA-dependent RNA polymerase* (*RdRp*) gene. This makes it highly unlikely that previously reported recombinant variants were overlooked in our analysis. Recombinant variants have been reported in which

the capsid protein genes from the 5′ region of the DWV-B genome are combined with the non-structural genes from the 3′ region of the DWV-A genome., found to be particularly prevalent in colonies experiencing high *Varroa* infestation [50,51]. Because the primers used in this study target regions specific to DWV-A, it is possible that such recombinant variants were amplified and therefore classified as DWV-A in our dataset. However, it cannot be completely ruled out that some colonies identified as positive for DWV-B may harbour a previously undescribed DWV-A/B recombinant, characterised by the presence of DWV-B sequences in both the 5′ and 3′ regions of the genome corresponding to the binding sites of the primers used in this study [7]. Furthermore, considering the recent identification of a new recombination breakpoint within the highly conserved *helicase*-coding region [52], the presence of recombinant viruses in some of the analysed colonies cannot be entirely excluded.

The spatio-temporal models generated in this study reveal a striking antithetical pattern in the seasonal dynamics of the two DWV variants. DWV-B exhibits a clear autumnal peak in November, followed by a decline during winter. This temporal profile coincides with the seasonal increase in *Varroa* populations, as brood rearing slows and mites concentrate on adult bees, enhancing mite-borne viral amplification and horizontal transmission [4,57]. In addition, the winter decline in DWV-B abundance may also reflect the impact of anti-*Varroa* treatments typically applied during this period in Italian apiaries. Such treatments, often based on oxalic or formic acid, are known to substantially reduce mite loads [38,40,58], thereby limiting virus transmission within colonies and explaining the observed drop in DWV-B prevalence after November. In contrast, DWV-A reaches its highest prevalence between June

and September, during the period of maximum foraging activity. The genotype DWV-B appears to outcompete DWV-A to such an extent that DWV-A is detected only when DWV-B occurs at low levels. Such a pattern suggests that DWV-A is less dependent on mite dynamics and may instead exploit environmental or trophallactic transmission pathways, such as virus-contaminated flowers, shared pollen and honey, or oral–faecal routes [55,59]. The inverse temporal relationship observed between the two DWV variants implies a complementary epidemiological strategy, where DWV-A dominates during periods of intense environmental interaction, while DWV-B capitalises on *Varroa*-driven transmission within colonies later in the season. This seasonal alternation may contribute to the long-term co-circulation and persistence of both genotypes across different ecological and management contexts.

Bayesian spatio-temporal regressions identified apiary density as one of the most influential covariates in differentiating DWV-A and DWV-B dynamics. DWV-B prevalence increased significantly with apiary density, highlighting its strong dependence on managed beekeeping intensity and the high inter-colony contact typical of dense apiaries. Such conditions facilitate horizontal viral spread via drifting bees, robbing behaviour, and mite transfer between hives [5,35]. Conversely, DWV-A showed a negative association with apiary density, which supports the hypothesis that this genotype may now persist and spread more effectively in low-density or semi-natural environments, likely through environmental or interspecific transmission routes [26,45,46,60,61]. This divergent behaviour between DWV-A and DWV-B may suggest a fundamental ecological differentiation between a *Varroa*-associated variant (DWV-B) and a more environmentally distributed variant (DWV-A). If the DWA-A is adopting an

alternative transmission route, it could represent an evolutionary response to displacement by the newer and more virulent variant B. Considering that sampling was conducted exclusively on adult bees, these results suggest that DWV-A may be more closely associated with the brood stage, whereas DWV-B appears to be more strongly associated with the adult stage, when mites concentrate on adult bees and benefit from active replication within the mite.

Land-use variables further clarify the distinct ecological niches occupied by the two DWV variants. For DWV-B, positive associations were detected with arable land compared to heterogeneous agricultural areas, suggesting that DWV-B may benefit from intensively managed agricultural systems, possibly reflecting the widespread use of honey-bee colonies for pollination services in these landscapes. On the other hand, arable lands include crops (such as regular annual cultivation, industrial flower crops, or aromatic and medicinal cultivation), which are characterised by synchronised blooms that attract numerous pollinators and thus could promote the spread of this DWV variant [62]. In contrast, DWV-A exhibited a weak yet positive relationship with permanent crops, as well as forest, semi-natural, and wet areas, but not with arable land. This pattern suggests that DWV-A may be less uniformly distributed across heterogeneous agricultural landscapes. Overall, these environmental associations are consistent with emerging evidence that DWV variants exploit distinct ecological networks. Nevertheless further research is required to elucidate the mechanisms underlying these patterns to highlight whether this environmental segregation could be due to a replacement process by DWV-B against DWV-A [2], or whether it may be the result of an evolutionary mechanism leading to different transmission routes for DWV-A.

The geographic distribution of DWV variants across Italy reveals a clear interplay between ecological, climatic, and management factors shaping viral epidemiology. The dominance of DWV-B in southern and insular regions appears closely linked to the mild and relatively stable Mediterranean climate, which allows *Varroa* populations to remain active for extended periods and limits natural brood breaks during winter [39,40]. In these conditions, the continuous availability of brood provides a persistent substrate for mite reproduction, promoting sustained *Varroa*-mediated viral amplification and facilitating the year-round maintenance of DWV-B within colonies. Such an environment, characterised by higher temperatures and prolonged foraging seasons, likely enhances both horizontal and vertical transmission of DWV-B, reinforcing its prevalence in managed apiaries located in warmer regions. Climatic influences on DWV dynamics have been demonstrated in several recent studies, showing that thermal extremes exert opposing effects on honey bee physiology and DWV replication [61,63,64]. Similarly, DWV infection intensity in pupae was demonstrated to be host-driven and temperature-dependent, with mid-range temperatures favouring viral replication and extremes reducing it [64]. Together, these studies highlight the narrow thermal window that optimises both host survival and viral persistence, providing a mechanistic explanation for the observed latitudinal gradient in DWV-B abundance. Large-scale epidemiological analyses corroborate this pattern, identifying clear regional and climatic predictors of honey bee virus prevalence across diverse environments, showing that warmer and less seasonal climates are consistently associated with higher viral incidence [65,66]. The concordance between their findings and the Italian data supports the

interpretation that DWV-B benefits from stable, warm conditions, which favour continuous *Varroa* activity and uninterrupted viral transmission cycles. By contrast, DWV-A prevalence was higher in central and northeastern regions, where seasonality seems to have a heavier impact on this variant compared to DWV-B, potentially enhancing environmental transmission routes, driven by more variable conditions [67].

The complementary temporal, spatial, and ecological distributions of DWV-A and DWV-B suggest ongoing viral niche partitioning and possibly competitive co-existence within the DWV complex. The contrasting distribution patterns observed between DWV-A and DWV-B suggest an ongoing ecological divergence within the DWV complex. It could be plausible that DWV-A, increasingly outcompeted in managed honey bee colonies by the more transmissible DWV-B, is becoming restricted to narrower ecological niches, potentially maintained within wild bee populations or other arthropods. Such hosts could act as environmental reservoirs, supporting low-level viral circulation independent of colony dynamics, thereby ensuring the long-term persistence of DWV-A in the wider pollinator community [46,59,68,69] or causing spillback phenomena from wild host to managed honey bee [70,71]. DWV-B's strong association with *Varroa* and high-density apiaries exemplifies a host–vector–pathogen co-adaptation [36], while DWV-A appears to persist through low-intensity, environmental transmission loops. Given the increasing frequency of recombinant genomes and adaptive interactions among DWV variants [33,37], these dynamics may evolve further, influencing not only viral prevalence but also colony health outcomes and interspecies spillover risks. Although *V. destructor* is widely recognised as a major driver of DWV

epidemiology, mite infestation levels were not explicitly quantified in this study. Future work integrating simultaneous monitoring of DWV variants together with *Varroa* infestation dynamics will therefore be important to clarify the relative contribution of environmental versus vector-mediated transmission pathways. Adding information on mite pressure alongside landscape composition and land-use factors would also improve the knowledge to understand and predict shifts in DWV genotype prevalence under changing climatic conditions and evolving beekeeping practices.

Conclusions

Overall, the results from this three-year national monitoring program reveal a clear dominance of DWV-B, tightly associated with high apiary density and managed landscapes. In contrast, DWV-A exhibits an opposite and complementary pattern, peaking during active foraging periods and showing links to environmental and diffuse transmission pathways. These contrasting ecological strategies illustrate how viral adaptation to anthropogenic and natural pressures can shape pathogen coexistence in complex pollinator systems, underscoring the importance of integrating epidemiological, ecological, and land-use perspectives in future honey bee health surveillance.

Materials and Methods

Sampling

The present study was carried out within the national monitoring project BeeNet [72], which involved 380 apiaries distributed across all Italian regions. Each apiary comprised three distinct honey bee colonies, monitored over three years (June 2021 – March 2024). Samples for pathological analysis were collected four times per year: in June (late spring–summer), September (late summer–autumn), November (autumn), and March (spring), following established protocols [73–75]. For each colony, approximately 25 forager bees were collected from the peripheral frames, where these individuals typically aggregate [38,76,77]. Samples were transported to the laboratory on dry ice and subsequently stored at −80 C until further analysis.

Extraction of nucleic acids

From each sample, a subsample of ten bees was randomly selected and processed as a pooled unit. The bees were placed in a 2-ml microcentrifuge tube containing 500 µl of DNA/RNA Shield (Zymo Research, Irvine, CA, USA) and homogenised for 3 minutes at 30 Hz using a TissueLyser II (Qiagen, Hilden, Germany), as previously described [45,46]. Nucleic acid extractions were performed using the Quick-RNA Microprep Plus Kit (Zymo Research), following the manufacturer's protocols adapted for solid tissue samples. Extracted nucleic acids were eluted in 100 µl of RNase-free water and stored at −80 °C until quantitative PCR (qPCR) analysis.

Quantitative real-time PCR (qPCR) assays

Quantitative real-time PCR (qPCR) essays were employed to assess the abundance of the two different variants of DWV. RNA samples were analysed for DWV-A and DWV-B, following specific primer pairs for DWV-A and DWV-B

(Table S3) [78]. Each qPCR reaction had a final volume of 14 µL, comprising 11 µL of master mix and 3 µL of RNA samples. The Power SYBR™ Green RNA-to-CT™ Kit (ThermoFisher Scientific) was used for RNA amplification. Reactions were run on a QuantStudio™ 3 Real-Time PCR System (ThermoFisher Scientific), with primer-specific annealing temperatures applied according to published protocols. The upper cycle threshold (Ct) of 35 for positive DWV-A and DWV-B detection was applied to minimise the risk of false positives [79]. Positive controls consisted of RNA extracted from previously confirmed infected honey bee samples. Sterile water was included as a negative control in all qPCR analyses. All assays were performed in duplicate. For absolute quantification, standard curves were generated for each target gene using serial dilutions of recombinant plasmids containing pathogen-specific RNA fragments, ranging from 1×10^{1} to 1×10^{10} copies per reaction [45,46], using the previously mentioned thermal protocol [78]. Data of standard curves are reported in Table S3.

Descriptive analyses

Statistical descriptive analyses were performed to assess both the prevalence and abundance of the two target viruses. Prevalence was defined as the proportion of positive samples over the total number of samples analysed, expressed as a percentage. Abundance was calculated as the mean of the two qPCR replicates and subsequently $\log_{10}$-transformed to normalise the data distribution. We reported prevalence with 95% confidence interval and mean, and standard deviations of copy numbers. The following analyses are conducted at the apiary level, rather than at the colony one. We considered one apiary as

positive to virus presence (either variant A or B) if at least one of the three colonies was.

Bayesian geostatistical models

Let Y_i a binary random variable (1/0) that indexes the presence/absence of DWV in the i^{th} apiary ($i = 1,...,380$) and let it follow a Bernoulli distribution with parameter π_i:

$$Y_i \sim \text{Bernoulli}(\pi_i)$$

where π_i denotes the probability of DWV variant. A Bayesian spatial Gaussian prior with an exponential covariance structure was specified for the random effects in the linear predictor of a probit model for the probability of infection. Specifically:

$$\text{probit}(\pi_i) = \Phi^{-1}(\pi_i) = \beta + z_i$$

where $\Phi^{-1}(.)$ is the Normal cumulative distribution function. The term z_i is the i^{th} component of a multivariate Gaussian vector with zero mean and variance-covariance matrix $\Sigma_{ij} = \sigma^2 \exp(-\varphi d_{ij})$, where σ^2 is assigned a Gamma (0.05, 0.005) prior distribution, d_{ij} is the Euclidean distance between apiaries i and j, and φ is the parameter controlling the decay of correlation with distance. We fixed the φ parameter in such a way that the correlation between points is 0.99 at minimum distance (200 m) and 6.34×10^{-20} at maximum distance (1473.47 km). The β intercept is assigned a weakly informative normal prior distribution. A Bayesian kriging was performed to predict the probability of infection in 617 unknown points which represent the centroid of the cells of a

regular 35 km grid over Italy. We run this model separately for each observed time point, producing 12 single prediction maps, for DWV-A and DWV-B.

Bayesian spatio-temporal model

We then specify a Bayesian spatio-temporal model to borrow strength information across time. In particular, we assume that $Y_{it} \sim \text{Bernoulli}(\pi_{it})$, where i indexes apiaries (i.e., space) and t denotes the time of sampling, therefore we have:

$$\text{probit}(\pi_{it}) = \Phi^{-1}(\pi_{it}) = \beta + z_i + \delta_t$$

where the term δ_t is the temporal random effect modelled a priori as Gaussian conditional autoregressive process of order two, i.e.:

$$\delta(t) \sim N(\bar{t}, \lambda_t n_t)$$

where $\bar{t}$ is the mean of the $(t - 2)^{th}$ and $(t + 2)^{th}$ terms, λ_t is the precision (assigned a $\text{Gamma}(0.05, 0.005)$ hyperprior), and n_t is the number of adjacent time points. Also in this case, we run the model separately for DWV-A and DWV-B. For the computational details, refer to the methods described in [80,81].

Bayesian spatio-temporal model with covariates

In order to assess the influence of the environment on variant spreading, we included in the Bayesian spatio-temporal model as covariates the density of apiaries per km² (calculated on the municipality level), and five variables indicating the type of landscape where the apiary was located. The density of apiaries was extracted from the “Beekeeping registry statistics dashboard” of the Italian Ministry of Health (https://www.vetinfo.it/j6_statistiche/#/report-pbi/45, retrieved on 31/12/25).

The landscape characterisation was carried out with the open-source geographic information system Q-GIS v 3.16.10. A circular buffer with apiary set as centroid and a radius of 1.5 km was calculated as a representative range for the forager bee activity [82–84] and extracted by the CORINE Land Cover layer (CLC) (https://land.copernicus.eu/en/products/corine-land-cover, retrieved on 31/12/25). Areas were then grouped into five distinctive categories:

- Artificial surfaces (category 1 CLC), consistent in areas artificially developed for human use, including residential and public buildings, industrial and commercial zones, transport infrastructures and green spaces such as parks.
- Arable lands (category 2.1 CLC), including land used in crop rotation for annual or fallow plants, rain-fed or irrigated.
- Permanent crops (category 2.2 CLC), such as lands with permanent, non-rotating crops such as olive groves, vineyards, and other perennial orchards.
- Heterogeneous agricultural areas (category 2.4 CLC), or areas where annual and permanent crops coexist or alternate with meadows, pastures, and natural vegetation.
- Forests, seminatural areas (category 3 CLC) and wetlands and water bodies (category 4 and 5 CLC), areas that include forested and wooded habitats with coniferous, broad-leaved and mixed forests and natural open zones such as grasslands. This category also comprises wetlands with low shrub, semi-woody or herbaceous vegetation, but also natural freshwater bodies such as lakes, ponds and rivers as well as artificial freshwater bodies like reservoirs and canals.

Since these covariates were expressed as percentages, they summed to one for each set of coordinates (i.e., for each apiary) and therefore constituted compositional data. Including all of them simultaneously in a regression model would lead to an identifiability issue. For this reason, we applied a log-ratio transformation - specifically an additive log-ratio (ALR) transformation [85] - after imputing zero values in the covariates through the count zero multiplicative (CZM) method. The component chosen as the reference category was “Heterogeneous agricultural areas”, selected solely because it was the most common one (86% of apiaries had a value greater than zero). As a consequence of the ALR transformation, this land-cover category does not appear explicitly in the model; all coefficients of the remaining land categories must therefore be interpreted with respect to it. Their coefficients are assigned weakly informative Normal prior distributions.

OpenBUGS [86] and R version 4.4.2 (r-project.org) were used for the analyses.

Acknowledgements

Data elaboration was supported by techniques acquired during the Master’s program in *‘Geostatistics for Human, Animal, and Environmental Health*’ offered by the University of Padua.

Additional Information

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.

This study was supported by the project BeeNet (Italian National Fund under FEASR 2014-2020) from the Italian Ministry of Agriculture and Food Sovereignty and Forestry (MASAF).

Data Availability Statement

The dataset used in the present study is available from the authors upon request.

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